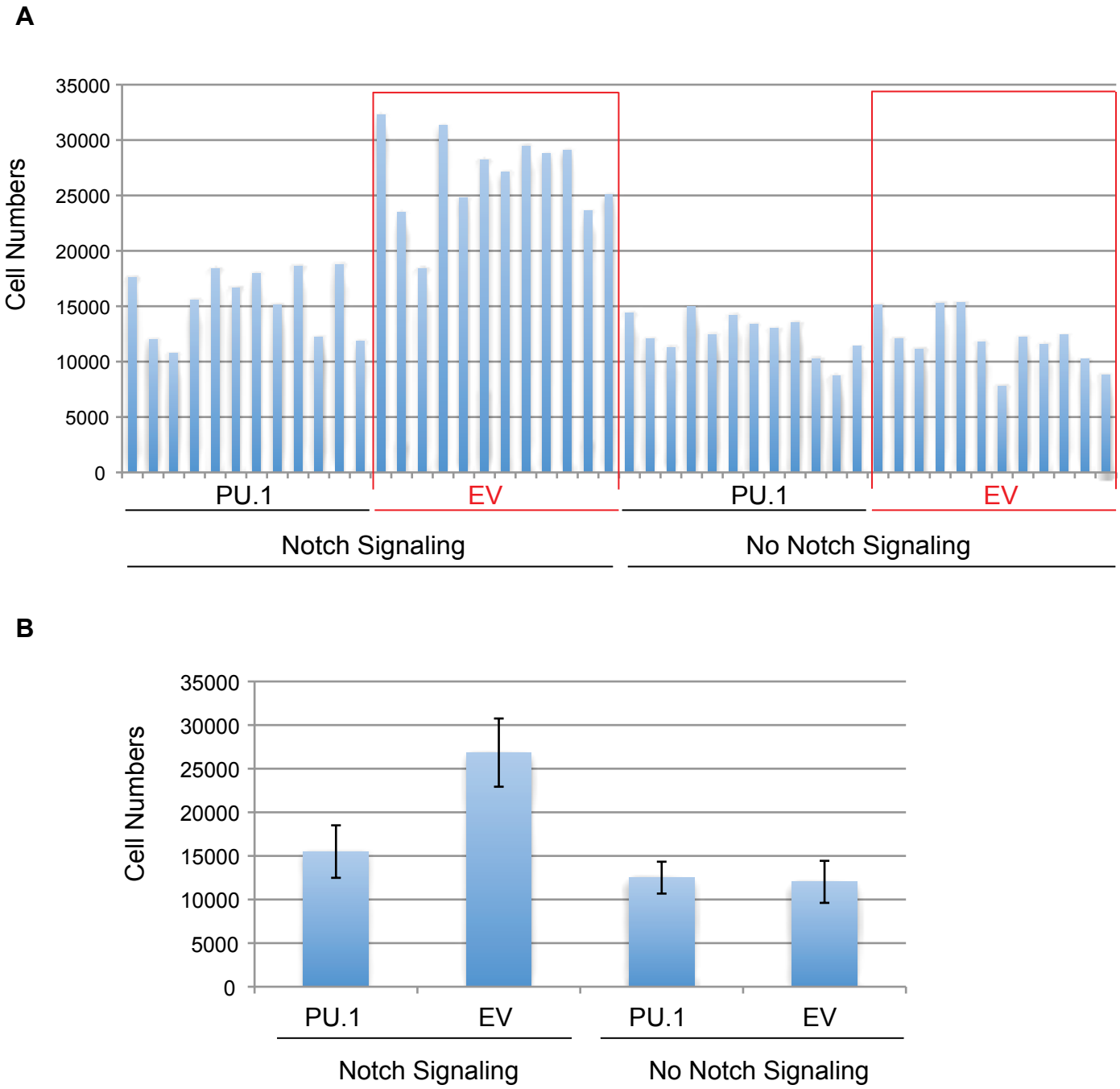

Fig. S1. Conditions for transcription factor perturbation used here minimize toxicity of high-level PU.1 in fetal thymocytes: PU.1 overexpression in Bcl2tg fetal thymocytes generates similar cell numbers compared with cells cultured in the absence of Notch signaling. E15.5 Bcl2tg fetal thymocytes were infected with PU.1 or empty vector for 4 hours and then cultured in the presence or absence of Notch signaling for 48 hours. Twelve different fetal bovine sera were used to make 12 culture media, thus representing 12 different cell culture conditions. (**A**) Fetal thymocyte cell numbers after 48 hours of transfection with PU.1 or empty vector under the conditions used for this study, then cultured with or without Notch signaling for 48 hours. Each bar represents a different cell culture condition within that group of samples. (**B**) The average cell number from the 12 different culture conditions in A. These results show that although enforced expression of PU.1 decreases cell output, this decrease is moderate and similar to culturing the cells in the absence of Notch signaling.

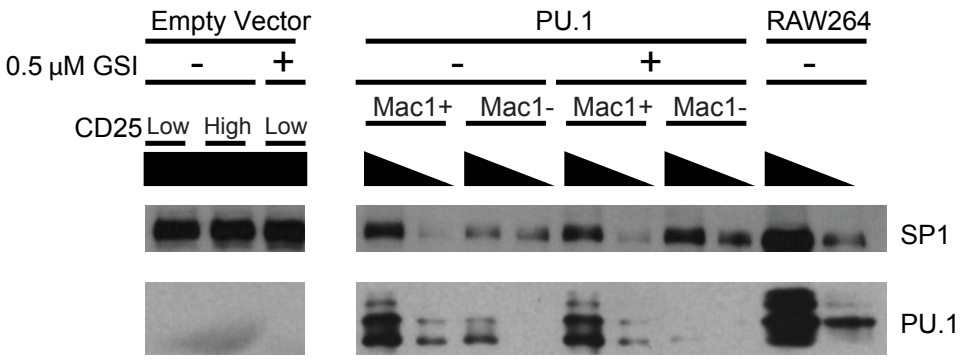

Fig. S2. Inhibition of Notch signaling in Scid.adh2c2 cells lowered the PU.1 dose threshold for the cells to divert to a Mac1$^+$ state, but did not alter PU.1 protein patterns. Scid.adh.2c2 cells were infected with PU.1 or empty vector and cultured in the presence or absence of γ secretase inhibitor (GSI) for 48 hours. The cells were then sorted according to their expression of Mac1 and lysed for western blot analysis, each sample assayed at 1× and 0.2× concentration with the decreasing concentrations indicated by wedge symbols over the lanes. The western blot was probed with anti-PU.1 antibody and antibody against the ubiquitous transcription factor SP1 as a loading control. The RAW264.7 macrophage cell line was used as a positive control for normal myeloid levels of PU.1 relative to SP1. The figure shows that cells with detectable PU.1 levels can remain Mac1$^-$ in the absence of GSI but not in the presence of GSI. The hierarchy of PU.1 band strengths, representing different post-translational modifications, is different in the myeloid cells from that in the transfected Scid.adh.2c2 cells. However, Mac1$^-$ and Mac1$^+$ Scid.adh.2c2 cells have the same band patterns, in the presence or absence of GSI.

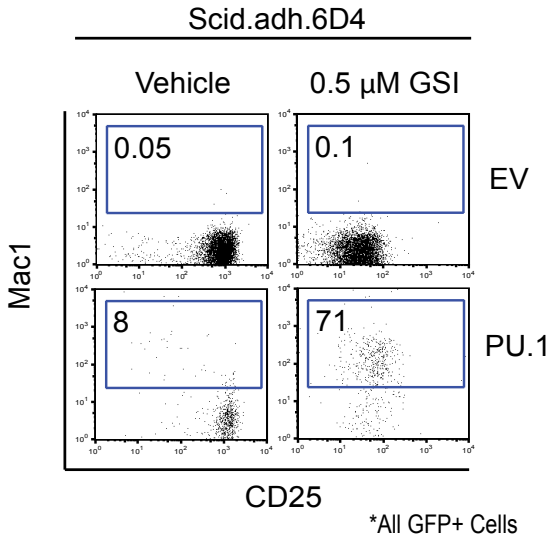

Fig. 3. A diversion-resistant subclone of Scid.adh cells becomes susceptible to diversion by PU.1 if endogenous Notch signaling is inhibited. Scid.adh.6D4 cells were transduced with empty vector or PU.1, and then cultured in the presence or absence of 0.5 μM GSI for 2 days as in Fig. 3A. Analysis shows the lack of Mac1 upregulation in these cells in response to PU.1 alone, but the efficient induction of Mac1 by PU.1 when Notch signaling is inhibited. These cells experience reduced viability in response to forced expression of PU.1 (Dionne et al., 2005), but the treatment with GSI is no more toxic to them than the effects of PU.1 alone.

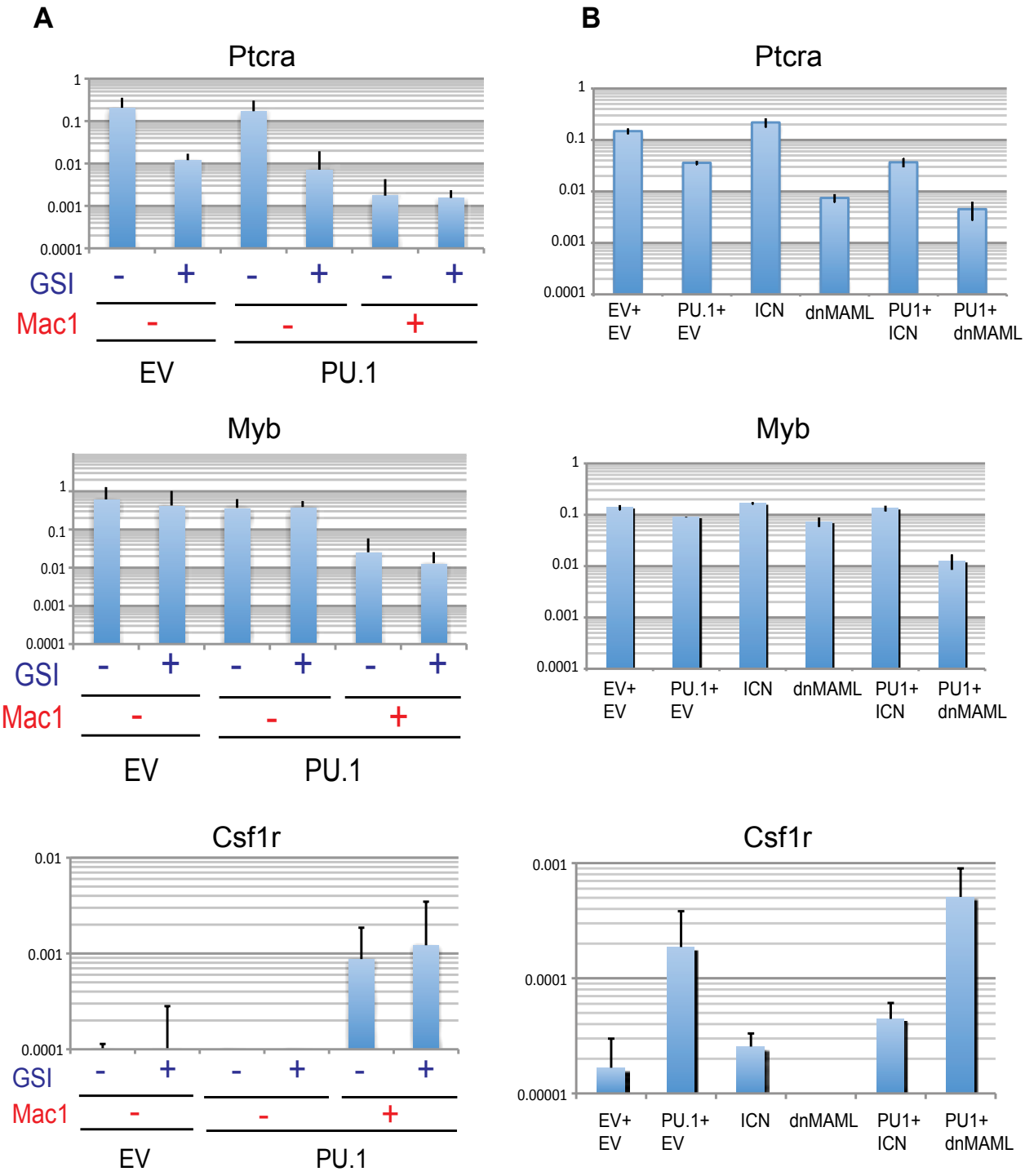

Fig. S4. Quantitive gene expression analysis using Scid.adh.2c2 cells. This figure illustrates examples of the gene expression levels and measurement errors among independent biological replicates for genes in the heat maps shown in the main text. (**A**) Scid.adh.2c2 cells transduced with PU.1 or empty vector and cultured in the presence of absence of GSI for 48 hours were sorted according to their expression of Mac1. The samples were then prepared for qRT-PCR analysis, with results summarized in Fig. 3C. Here we show 3 representative bar graphs showing gene expression levels of Ptcra, Myb and Csf1r. Geometric means from four independent biological replicates of the full sets of experimental samples are shown ± SD. (**B**) Representative bar graphs of gene expression in sorted Scid.adh.2c2 cells co-expressing PU.1 and empty vector, ICN, or dnMAML after 48 hours, as summarized in Fig. 4B. *Ptcra* was down regulated in response to decreased Notch signaling and/or PU.1 transduction. *Myb* was down regulated only in response to diversion. *Csf1r* was up regulated only in response to diversion. Four independent biological replicates of the full sets of experimental samples were analyzed.

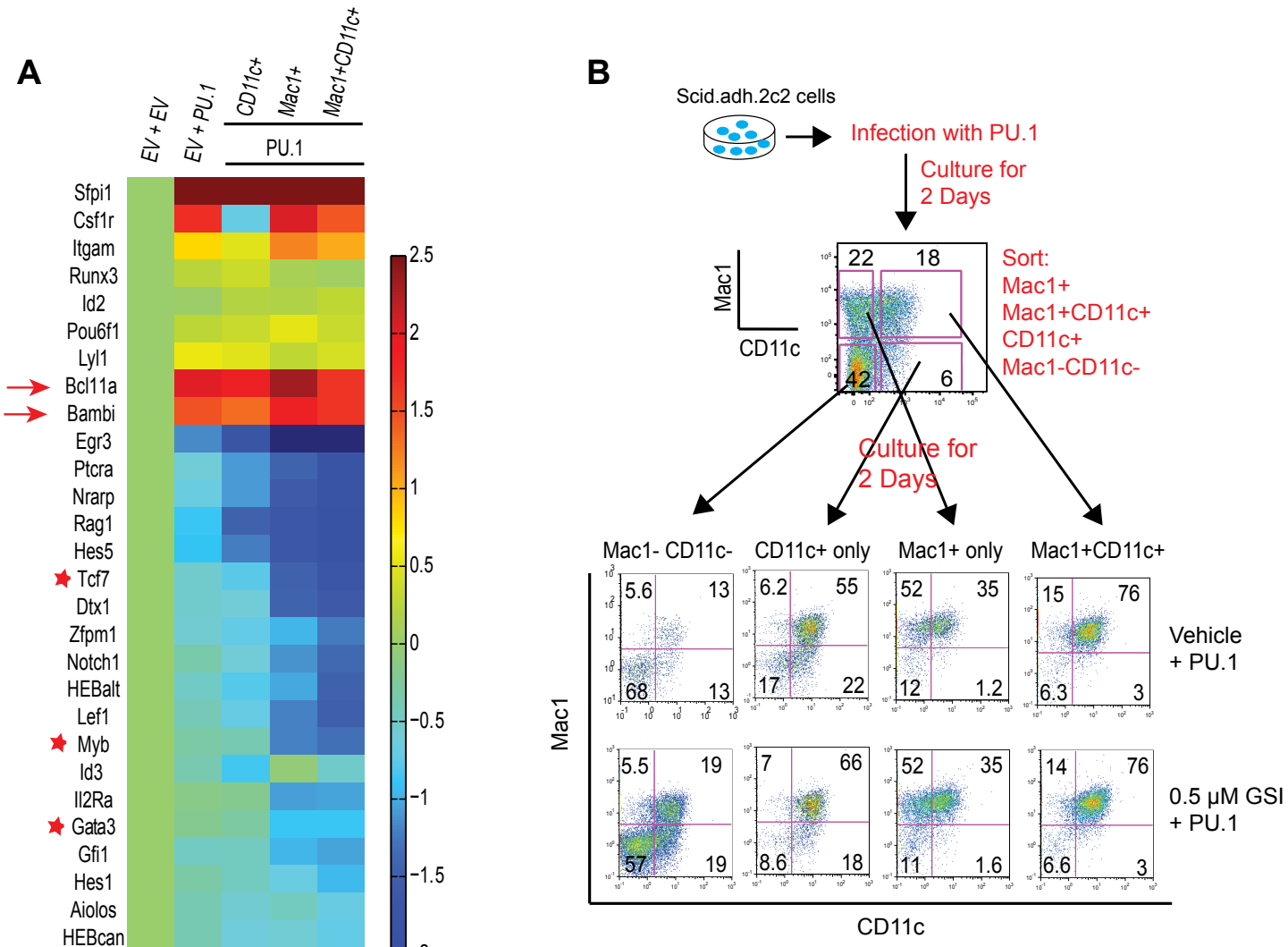

Fig. S5. Mac1 up-regulation in Scid.adh2c2 cells accompanies a more severe repression of T cell genes such as *Tcf7, Myb and Gata3* when compared to the up regulation of CD11c. (**A**) Scid.adh.2c2 cells were infected with PU.1 or empty vector and cultured for 48 hours. The cells were then sorted according to their expression of the viral vector and the expression of Mac1 and/or CD11c. QRT-PCR analysis was performed on the samples. As shown in Fig. 4A, the up-regulation of these two markers is not completely coordinate, and Notch signals appear more effective at blocking Mac1 expression than at blocking CD11c expression. Gene expression results in panel A show that repression of T-cell genes and activation of *Csf1r* are not, in fact, complete in the CD11c+ Mac1– cells. (**B**) Cells with CD11c+ Mac1– phenotype can be intermediates toward full diversion. Schematic shows the experimental plan. After sorting distinct subsets of transduced Scid.adh.2C2 cells based on their patterns of CD11c/Mac1 expression at 2 days (middle panel), further culture for 2 days more with or without GSI reveals that CD11c+ Mac1– cells are primed to progress to a fully diverted CD11c+ Mac1+ phenotype.

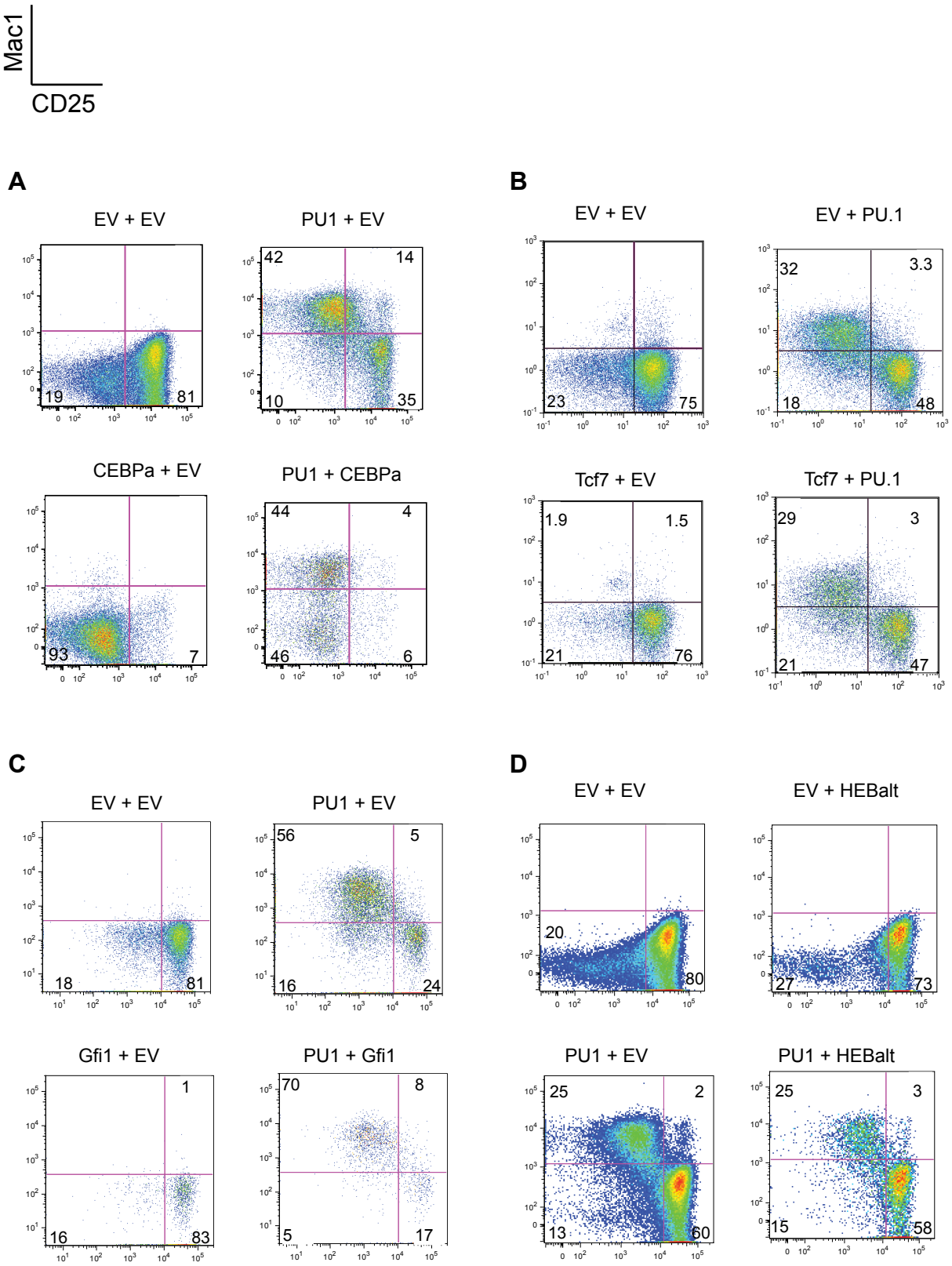

Fig. S6. Scid.adh.2c2 cells co-expressing PU.1 with *Tcf7*, *Cebpa*, or *HEBalt* do not alter their expression of Mac1 in comparison to cells expressing PU.1 alone, while co-expression of PU.1 with *Gfi1* increases the percentage of Mac1+ cells compared to PU.1 alone. (**A-D**) Scid.adh2c2 cells were infected with (A) *Cebpa*, (B) *Tcf7*, (C) *Gfi1* or (D) HEBalt (*Tcf12*, alternative promoter isoform) for 24 hours. The cells were then infected with PU.1 or empty vector and cultured for an additional 48 hours. The doubly infected cells were analyzed for their expression of Mac1 and CD25 using flow cytometry.

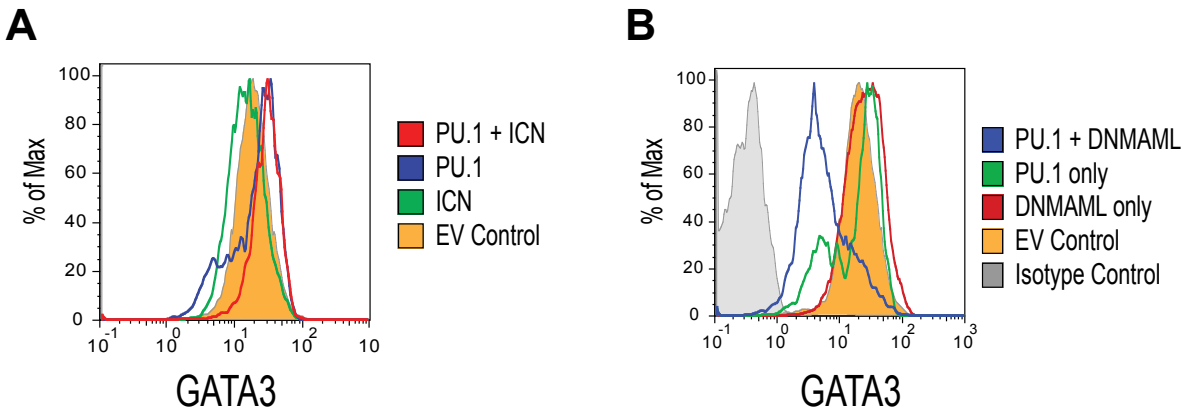

Fig. S7. GATA3 protein levels are decreased by PU.1 in the absence of Notch signaling, but are unchanged by PU.1 in the presence of high levels of Notch signaling. (**A**) Scid.adh.2c2 cells were infected with PU.1 and ICN, and cultured for 48 hours. GATA3 intracellular staining shows that PU.1 alone can downregulate GATA3 protein in some cells, whereas PU.1 co-expressed with ICN and ICN alone do not downregulate GATA3 protein levels. (**B**) Scid.adh2c2 cells infected with PU.1 and dnMAML for 48 hours show increased downregulation of GATA3 proteins levels compared with cells expressing only PU.1. dnMAML alone does not downregulate GATA3 protein levels.

Download Table S1

Table S1. Actual measured RNA expression levels in thymocytes 16 hours after transduction with PU.1 or empty vector. (**A**,**B**) DN2 (A) and DN3 (B) thymocytes. Results shown are averages from two or three independent experiments, and are given in units relative to *Actinb* expression levels in the samples. Delta, culture on OP9-DL1; Mig, culture on OP9-control ('OP9-Mig'). (**C**) Genes showing significant effects of PU.1 and/or interaction with Notch signaling, based on two-way ANOVA analysis.

Download Table S2

Table S2. Compilation of actual gene expression values from all Scid.adh.2c2 perturbation experiments in Figs 3-7. (A-E) Individual worksheets provide average values for the measured genes, in units relative to *Actinb*, from 2-4 independent experiments per analysis. (**A**) Gene expression effects during PU.1-mediated diversion to Mac1^{+}, in presence or absence of GSI. Patterns of response are summarized in tabular form in main Fig. 3D. (**B**) Gene expression effects of empty vector or PU.1 together with forced Notch activation (ICN1) or forced Notch inhibition (dnMAML). A summary of distinct patterns of response is shown to the right of the columns of measured gene expression values, and referred to by 'response group' number in the text. (**C**) Gene expression effects of PU.1 together with Id2. A summary of distinct patterns of response is tabulated on the right as in worksheet B. (**D**) Gene expression effects of PU.1 together with Myb. A summary of distinct patterns of response is tabulated on the right as in worksheet B. (**E**) Gene expression effects of PU.1 together with Gata3 shRNA. A summary of distinct patterns of response is tabulated on the right as in

Table S3. Primer sequences for quantitative real-time RT-PCR

Gene	Forward	Reverse
Ski	ACTACACGGGCAAGGAGGAG	TGGACTGCTTGTCCTTCTCA
Smad3	TGTCCCCAGCACACAATAAC	GTAAGTTCCACGGCTGCATT
TGFBR1	AGAAGAGCGTTCATGGTTCC	AATCTGACACCAACCACAGC
IL7Ra	TGGCTCTGGGTAGAGCTTTC	GTGGCACCAGAAGGAGTGAT
Hck	AGTCCAGGTTCCTCCGAGAT	GCTGTTGCTGTTGTTTGGTC
Bambi	CGTCCTCTCTCCTTCCAAGA	GGAAGTCAGCTCCTGCATCT
Limd1	CTCCACTGCGGAATTGAAGT	AAGCACGCATCGTGGTAGAG
Psen1	ACTTCCAGAATGCCCAGATG	TGTTCCACCACCTGTCTTGA
Psen2	GGAGGATGGAGAGAGCACTG	TGCCCGTTCTTCTCAGTGTA
Zfp710	CACTGAGTGTGGCATGGAGT	TCTTGAGGGTTTGCTTCTGC
Fes	GCAAACTGAGCGTGCTGAT	TACCAGGGTCCGGTACTGAG

Other primer sequences used have been previously described (David-Fung et al., 2009; Li et al., 2010; Yui et al., 2010).